8th Edition, revised in February, 2018

AMOZ (Furaltadone Metabolite) ELISA Kit

Catalog No: E-FS-E002

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect AMOZ in samples, such as honey, fish, shrimp, fowls, liver, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with coupled antigen. During the reaction, AMOZ in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti- AMOZ antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of AMOZ. The concentration of AMOZ in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.05 ppb (ng/mL)

Reaction mode: 25℃, 45 min~15 min

Detection limit: (Pig, chicken, duck) Tissue/liver ---0.1 ppb, Honey/milk ---0.1 ppb,
Milk powder/egg powder/feed---0.1 ppb,
Fish/shrimp---0.15 ppb (The quantitative detection limit of fish, shrimp and other aquatic products is 0.15 ppb due to some interferences.)

Cross-reactivity: Furaltadone metabolite---100%, Furazolidone metabolite---＜0.1%,
Nitrofurantoin metabolite---＜0.1%, Nitrofurazone metabolite---＜0.1%

Sample recovery rate: Tissue/liver ---80%±25%, Honey/milk --75%±15%,
Milk powder/egg powder/feed---85%±25%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
Derivatization Reagent	10 mL
HRP Conjugate	5.5 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
2×Reconstitution Buffer	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other supplies required

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen evaporators/Water bath Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: Single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

Reagents: Ethyl acetate, n-hexane, methyl alcohol, Acetonitrile, NaOH, concentrated HCl, $K_2HPO_4 \cdot 3H_2O$, $Na_2Fe(CN)_5(NO) \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:** experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

Solution 1: 0.36 M $Na_2Fe(CN)_5(NO) \cdot 2H_2O$ solution (for milk and milk powder sample)

Dissolve 10.7g M $Na_2Fe(CN)_5(NO) \cdot 2H_2O$ to 100 mL with deionized water.

Solution 2: 1.04 M $ZnSO_4 \cdot 7H_2O$ solution (for milk and milk powder sample)

Dissolve 29.8g $ZnSO_4 \cdot 7H_2O$ to 100 mL with deionized water.

Solution 3: 0.1 M K_2HPO_4

Dissolve 11.4g $K_2HPO_4 \cdot 3H_2O$ to 500 mL with deionized water.

Solution 4: 1 M HCl solution

Dilute 8.6 mL concentrated HCl to 100 mL with deionized water.

Solution 5: 1 M NaOH solution

Dissolve 4g NaOH to 100 mL with deionized water.

Solution 6: Reconstitution Buffer

Dilute the 2×Reconstitution Buffer with deionized water. (2× Reconstitution Buffer (V): Deionized water (V) =1:1). The Reconstitution buffer can be store at 4℃ for 1 month.

Solution 7: Wash Buffer

Dilute 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

3. Sample pretreatment

3.1 Pretreatment of milk (liquid):

(1) Take 5 mL of milk into 50 mL EP tube, add 250 μL of Solution 1 and oscillate for 30 sec, then add 250 μL of Solution 2 and oscillate for 30 sec, centrifuge at 4000 rpm for 10min at 15℃.

(2) Take 1.1 mL of supernatant, add 4 mL of deionized water, 0.5 mL of Solution 4 and 100 μL of **derivatization reagent**, oscillate for 5 min.

(3) Incubate overnight at 37℃(about 16 hours) or incubate in water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).

(4) Add 5 mL of Solution 3, 0.4 mL of **1 M NaOH solution** and 5 mL of ethyl acetate, oscillate for 5 min.

(5) Centrifuge at 4000 rpm at room temperature for 10 min.

(6) Take 2.5 mL of upper liquid to another tube, dry at 50-60℃ with nitrogen evaporators/water bath

(7) Dissolve the residual with 1 mL n-hexane, add 1 mL of **Reconstitution buffer** and oscillate for 30 sec. Centrifuge at 4000 rpm at room temperature for 10 min.

(8) Discard the upper n-hexane, take 50 μL lower liquid for analyze

Note: Sample dilution factor: 2, minimum detection dose: 0.1 ppb

3.2 Pretreatment of milk powder, egg powder:

(1) Weigh 1±0.05 g of sample into 50 mL EP tube, add 4 mL of deionized water, 0.5 mL of Solution 4 and 100 μL of **derivatization reagent**, oscillate for 5 min.

(2) Incubate overnight at 37℃(about 16 hours) or incubate with water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).

(3) Add 250 μL of Solution 1, oscillate for 30s, then add 250 μL of Solution 2, oscillate for 30 sec centrifuge at 4000 rpm at 15℃ for 10 min.

(4) Take the supernatant to another tube, add 5mL of Solution 3, 0.4 mL of **1 M NaOH solution** and 5 mL of ethyl acetate, oscillate for 5 min.

(5) Centrifuge at 4000 rpm at room temperature for 10 min.

(6) Take 2.5 mL of upper liquid to another tube, dry at 50-60℃ with nitrogen evaporators/water bath

(7) Dissolve the residual with 1mL n-hexane, add 1 mL of **Reconstitution buffer** and oscillate for 30 sec. Centrifuge at 4000 rpm at room temperature for 10 min.

(8) Discard the upper n-hexane, take 50 μL of lower liquid for analyze.

Note: Sample dilution factor: 2, minimum detection dose: 0.1 ppb

3.3 Pretreatment of honey, (pig, chicken, duck) tissue/liver, fish/shrimp, feed:

(1) Weigh 1±0.05 g of homogenate sample into 50 mL EP tube, add 4 mL of deionized water, 0.5 mL of Solution 4 and 100 μL of **derivatization reagent**, oscillate for 5min.

(2) Incubate overnight at 37℃ (about 16 hours) or incubate in water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).

(3) Add 5 mL of Solution 3, 0.4 mL of **1 M NaOH solution** and 5 mL of ethyl acetate, oscillate for 5 min.

(4) Centrifuge at 4000 rpm at room temperature for 10 min.

(5) Take 2.5 mL of upper liquid to another tube, dry at 50-60℃ with nitrogen evaporators/water bath

(6) Dissolve the residual with 1 mL n-hexane, add 1 mL of **Reconstitution buffer** and oscillate for 30 sec. Centrifuge at 4000 rpm at room temperature for 10 min.

(7) Discard the upper n-hexane, take 50 μL lower liquid for analyze

Note: Sample dilution factor: 2, minimum detection dose: 0.1 ppb

Note: Sample dilution factor: 2, quantitative detection limit of fish/shrimp: 0.15 ppb

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **Standard or Sample** per well, then add 50 μL of **HRP Conjugate** to each well, then add 50 μL of **Antibody Working Solution**, cover the plate with sealer, oscillate for 5 sec and mix thoroughly, incubate at 25℃ for 45 min.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 30 sec intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5 sec to mix thoroughly. Incubate at 25℃ for 15 min with shading light (The reaction time can be extended according to the actual color change).
5. **Stop reaction:** add 50 μL of **stop solution** to each well, oscillate gently to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance (%) =A/A_0 ×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.**

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. **Do not use expired kit, reagents of different batches and reagents that do not belong to this kit**.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit($A_{450\ nm}$＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and valid period

Store at 2~8℃ for 1 year. Avoid freeze.

Please store the opened kit at 2~8℃, protect from light and moisture. The valid period is 2 months.

Expiry date: expiration date is on the packing box.